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Influence of Copper on Selected Physiological Responses in *Salvinia minima* and Its Potential Use in Copper Remediation

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ABSTRACT.—This study was designed to evaluate selected physiological responses of *Salvinia minima* to copper (Cu^{2+}) concentrations of 0.06 (control), 1.0, 2.0, 2.5, and 3.0 mg l^{-1}. The plants were grown under laboratory conditions of 25 ± 2°C, a light intensity of 120 μmol m^{-2} s^{-1}, and a 14-h photoperiod. After seven days of exposure to the Cu, *Salvinia* growth decreased gradually with an increase in Cu concentration resulting in a significant decline at 3.0 mg l^{-1}Cu. Similar results were obtained after 14 days of exposure. However, calculating growth using fresh weight showed a significant decline at 2.5 and 3.0 mg l^{-1}. After 14 days exposure, CO_2 assimilation decreased as the Cu concentration increased in the growth media. This decrease in CO_2 assimilation coincided with a similar decrease in photosynthetic pigments. Uptake of Cu significantly increased with the increase of Cu concentration in the growth media. This study demonstrated the potential of *Salvinia* to remediate Cu in concentrations 100 times what is currently found in freshwater environments.

Toxic heavy metal contamination is common in aquatic ecosystems due to both anthropogenic and natural sources. Runoff, industrial waste discharge and sewage effluent are the most frequent anthropogenic sources of aquatic contamination (Lee *et al.*, 1998).

Copper (Cu), an essential metal for plant growth is required in trace amounts (Guilizoni, 1991). Copper is a constituent of the chloroplast protein plastocyanin, which forms part of the electron transport chain linking the two photochemical systems of photosynthesis (Bowyer and Leegood, 1997). In addition, Cu functions as an activator or component of certain enzymes that are involved in a variety of biochemical processes, such as cytochrome *c* oxidase, and Cu-Zn superoxide dismutase (Linder, 1991).

Copper uptake appears to be a metabolically mediated process and there is evidence that Cu strongly inhibits the uptake of zinc (Zn) and *vice versa* (Hawf and Schmid, 1967). Generally, Cu toxicity causes chlorosis (Lewis, 1993; Vavilin *et al.*, 1995) and iron (Fe) deficiency by inhibiting translocation of Fe through the plant (Chaney, 1970; Lingle *et al.*, 1963; Wallace and DeKock, 1966). In addition, toxic levels of Cu inhibit root growth by damaging plasma membrane integrity (Marschner, 1995).

Copper is one of 13 metals listed as a priority pollutant by the U.S. Environmental Protection Agency (EPA) (Salomons *et al.*, 1995) and is among one of the most frequently discharged elements into the environment. It has been estimated that the global discharge of copper in aquatic systems is near 112×10^3 metric tons per year (Moore, 1991). Conventional remediation

methods such as precipitation, chemical oxidation or reduction, ion exchange, filtration, or evaporation processes are generally inefficient for removing metals in aquatic systems (Bervoets *et al.*, 1994). In contrast, the use of aquatic plants is currently under investigation as a viable alternative for remediation of a wide range of contaminants including heavy metals (Lee *et al.*, 1998). This cost-effective, plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues (Salt *et al.*, 1998). Selecting plants as suitable candidates in phytoremediation must satisfy certain criteria such as reasonable tolerance to the contaminant in question, relatively high growth rate, and the ability to uptake and preferably metabolize the contaminant (Salt *et al.*, 1998).

The genus *Salvinia* (Salviniaceae) is comprised of one genus and 10 known species (Nauman, 1993). *Salvinia minima* Baker is a small, free-floating freshwater fern found in tropical and temperate regions of the world (DeBusk and Reddy, 1987) in areas such as North, South, and Central America, the West Indies, and Central America (Nauman, 1993). This plant can be found floating near the edges of slow moving streams and in nutrient enriched ponds. It is commonly referred to as water spangles and floating fern (Nauman, 1993). *Salvinia minima* demonstrated the ability to withstand aluminum (Al) concentrations of 20 mg l^{-1} through the manipulation of the media pH from 3.9 to near 7 within 24 hours (Gardner and Al-Hamdani, 1997). In addition, *Salvinia* showed considerable ability to accumulate cadmium (Cd II), 10,930 mg kg^{-1}; therefore it was suggested as a Cd II hyperaccumulater (Olguin *et al.*, 2002). *Salvinia* has the potential to double its population in approximately 3.5 days (Nichols *et al.*, 2000) making it a suitable candidate for phytoremediation.

This study was designed to evaluate the impact of Cu^{2+} concentrations of 0.06 (control), 1.0, 2.0, 2.5, and 3.0 mg l^{-1} on various physiological responses of *Salvinia* including plant growth, photosynthetic pigments, and CO_2 assimilation. The 0.06 mg l^{-1} concentration designated as the control was selected based on the average concentration of Cu in uncontaminated freshwater (Boyd, 1990). Copper uptake by *Salvinia*, grown at the different treatments, was determined.

Materials and Methods

The *Salvinia minima* utilized in this study was taken from stock material grown under greenhouse conditions for four years. Dr. David Whetstone, at the Jacksonville State University Herbarium, identified the plants, which were originally collected at a drainage ditch near Sanford, Florida (USA). Plants with a total of 15 fronds were placed into 60 (250 ml) Erlenmeyer flasks containing 125 ml of various Cu concentrations dissolved in 10% Hoagland solution with a pH of 6.5 (Hoagland and Arnon, 1938). Twelve flasks, samples, were used for each of the selected Cu concentrations, control (0.06); 1.0; 2.0; 2.5; and 3.0 mg l^{-1}. The initial fresh weight of the plants was recorded for each flask. The samples were placed randomly in the growth chamber and allowed to grow under conditions of 25 ± 2°C, a light intensity of 120 μmol m^{-2} s^{-1} and a 14-h

photoperiod. On day seven of the experiment, plant fresh weight and total frond number of six randomly selected flasks were assayed. In addition, 0.1 g fresh weight of tissue from each flask was used for chlorophyll *a* and *b*, and carotenoid determination. The remaining samples from these flasks were oven dried at 80°C for 48 hrs to be used later for Cu uptake determination. The media of the remaining six samples of each treatment were replaced with fresh solutions on day seven and the plants were allowed to grow for an additional seven days. On day 14 of the experiment, the same physiological parameters were determined as was CO_2 assimilation.

A separate experiment, as described above, was conducted with the exception that the media were not replaced at day seven. The existing media was filtered twice to reduce algal contamination. These plants were harvested on day 14 and oven dried at 80°C for 48 hrs to be used later for total Cu uptake. In addition, the medium of each sample was collected and the filters were analyzed for total Cu concentrations to insure a total accounting for Cu partitioning. The data obtained from this experiment was used to determine bioconcentration factors (BCF) and percent of Cu uptake.

Salvinia growth was expressed as doubling time (DT) in days. The doubling time was determined using the following equation: $DT = t \log 2 [\log (w_t w_o^{-1})]^{-1}$ (Moretti and Gigliano, 1988), where DT is the doubling time (days), t is the experiment duration (days), w_t is the final weight (or number of fronds), and w_o is the initial weight (or number of fronds).

Approximately 0.1 g fresh weight of each sample was used for measuring chlorophyll *a*, *b*, and carotenoid concentration. The plant was placed into 5 ml of N,N-Dimethylformamide (DMF) solution. The samples were incubated in the dark for 36 hrs at 4°C. Chlorophyll *a* and *b* was determined spectrophotometrically at wavelengths of 647 and 664.5 nm (Inskeep and Bloom, 1985). Carotenoid concentrations of the DMF extract were determined spectrophotometrically at a wavelength of 470 nm and the concentration was calculated using the formula of Doong et al. (1993).

Carbon dioxide assimilation and internal CO_2 concentrations of six randomly selected samples from each treatment were measured four hours after the onset of the light period on days seven and 14 of the treatments application. The selected plants of each sample were enclosed in a flow-through plexiglass assimilation chamber (4.5 by 11.8 by 7.3 cm) of a Li-Cor 6200 photosynthesis system (Lincoln, NE, USA) as described by McDermitt et al. (1989). Standard measurement conditions were 120 μmol m^{-2} s^{-1} photon flux density, 45 to 50% RH, and 25°.

Oven dried plant samples, ranging from 0.01-0.07 g, were digested according to procedures for Cu sampling outlined in the Buck Model 210 VGP Atomic Absorption Spectrophotometer Operating Manual (Buck Scientific, 1996). The samples were refluxed in 10 ml of 6N nitric acid for 15 min, just below the boiling point, and then 5 ml concentrated nitric acid (15.8N) was added. The reflux process was continued until the sample volume was reduced to approximately 5 ml. The samples were allowed to cool after which 2 ml H_2O and 5 ml 30% H_2O_2 were added to each sample. The samples were warmed

TABLE 1. Influence of different Cu concentrations on *Salvinia* growth. Plant growth is expressed as doubling time (DT) based on fresh weight and frond number for both seven and 14 days of exposure. LSD (P = 0.05) = Least Significant Difference value for difference between means within a column. Same letter denotes no statistical difference. Lower case letters denote differences between treatments within a day and upper case letters denote differences between days within treatments. N = 24

	DT based on mean frond number (days)		DT based on mean fresh weight (days)	
	Length of exposure (days)			
Cu (mg l^{-1})	7	14	7	14
0.06 (control)	7.61 aA	8.23 aA	5.70 aA	7.68 aB
1.0	8.37 abA	8.44 aA	6.03 aA	8.39 abB
2.0	8.10 abA	8.65 abA	6.83 aA	8.79 abB
2.5	8.34 abA	9.17 abA	7.87 abA	9.22 bB
3.0	9.84 bA	9.82 bA	8.92 bA	10.06 bB

slowly adding 1 ml of 30% H_2O_2 as needed until effervescence subsided. After cooling again, reflux of the samples was continued for 15 min. using HCl in aratio of 1 ml for each 2 ml of sample. After cooling, the samples were brought to 25 ml with distilled H_2O. Standards were established using Cu concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 mg l^{-1} and absorbance was measured for each sample using the atomic absorption spectrophotometer.

Copper concentrations in the growth media were determined following the digesting procedure outlined in the Buck Model 210 VGP atomic absorption spectrophotometer-operating manual (Buck Scientific, 1996). To the sample media, 2 ml nitric acid (15.8N) and 5 ml HCl was added. The samples were refluxed until approximately one quarter of the media remained and the volume brought back to 125 ml with distilled H_2O. The Cu concentration of each sample was determined as described above. The BCF was determined as the ratio of Cu concentration in the plant tissue to the concentration in the external media (Spacie *et al.*, 1995).

The experiments were statistically analyzed as a randomized complete block design (Steel and Torrie, 1980). This design ensured that observed differences in plant performances were largely due to treatments rather than variation among the four blocks. The block in this study represent the replicate series of each experiment conducted at different times. Mean separations for the treatments with significant F values (P = 0.05) of ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS

After seven days of exposure to the various Cu concentrations, the only significant reduction in *Salvinia* growth was obtained on the medium containing 3.0 mg l^{-1} Cu (Table 1). Similar results were obtained at the end of day 14 of treatments exposure. Additionally, using fresh weight to calculate DT, the data showed that Cu concentrations of 2.5 mg l^{-1} significantly reduced

TABLE 2. Concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in salvinia as influenced by different Cu concentrations after seven and 14 days. LSD (P = 0.05) = Least Significant Difference value for difference between means within a column. Same letter denotes no statistical difference. Lower case letters denote differences between treatments within a day and upper case letters denote differences between days within treatments. N = 24

	Chl a (mg g^{-1} fr wt.)		Chl b (mg g^{-1} fr wt.)		Carotenoid (mg g^{-1} fr wt.)	
	Length of exposure (days)					
Cu conc (mg l^{-1})	7	14	7	14	7	14
0.06 (control)	11.67 aA	12.82 aB	6.93aA	7.01 aA	3.14aA	3.61 aA
1.0	6.02 bA	5.98 bA	3.82 bA	3.09 bB	0.47 bA	0.37 bA
2.0	5.10 bA	3.67cB	3.25 cA	1.86cB	0.30 bA	0.07 bA
2.5	5.60 bA	4.36 cB	3.66bcA	2.40 dB	0.42 bA	0.16bA
3.0	5.29 bA	4.57 cB	3.35 bcA	2.43 dB	0.33bA	0.17bA

Salvinia growth at the end of day14 of treatment (Table 1). *Salvinia* growth, measured as frond number DT, was not significantly different in the presence of 1.0, 2.0, 2.5 mg l^{-1} Cu from that of 3.0 mg l^{-1} Cu. Comparing the same treatments, day 14 data analysis was shown that significant reduction in growth at 3.0 mg l^{-1} Cu in contrast to that of 1.0 mg l^{-1} Cu. Slight deviation from these findings was obtained from data analysis using plant fresh weight DT that reveled growth was significantly less in 3.0 mg l^{-1} Cu than at 1.0 and 2.0 mg l^{-1} Cu. Using frond number DT, no differences were noted in *Salvinia* growth between day seven and 14 at each of the various Cu concentrations. However, there was a significant increase in fresh weight DT values, which corresponds to a significant reduction in growth, at the end of 14 days for all treatments in comparison to those obtained at day seven (Table 1).

After seven days exposure, the increase in Cu concentrations from 1.0 to 3.0 mg l^{-1} had similarly influenced chlorophyll *a* and *b* and carotenoid concentrations and significantly reduced these photosynthetic pigments, relative to the control. The only exception to this finding was Chlorophyll *b*, which was significantly higher at 1.0 than at 2.0 mg l^{-1} Cu (Table 2). In general, a reduction in *Salvinia* photosynthetic pigments obtained at day 14 reflected that seen at seven days (Table 2). However, at 1.0 mg l^{-1} Cu, chlorophyll *a* concentration was significantly higher than that of the other treatments except for the control, 0.06 mg l^{-1}. The gradual decrease in chlorophyll *b* was interrupted by an increase in Cu from 2.5 to 3.0 mg l^{-1} in comparison to 2.0 mg l^{-1}. Chlorophyll *a* accumulation in *Salvinia* significantly increased at day 14 in comparison to day seven within the control (Table 2). However, chlorophyll *b* and carotenoid concentrations were not significantly affected. In comparison the photosynthetic pigments between days seven and 14 for each treatment, carotenoid concentrations showed no significant difference whereas *Salvinia* grown at Cu concentrations of 1.0 mg l^{-1} and higher revealed a significant reduction in chlorophyll *a* and *b*, with the exception of chlorophyll *a* at 1.0 mg l^{-1}.

After fourteen days of Cu exposure there were significant decreases in CO_2 assimilation for all Cu concentrations from 1.0 to 3.0 mg l^{-1} as compared to the

TABLE 3. Carbon dioxide assimilation and internal CO_2 in *Salvinia* after 14 days of varying Cu exposure. LSD (P = 0.05) = Least Significant Difference value for difference between means within a column. Same letter denotes no statistical difference. Lower case letters denote differences between treatments within a day and upper case letters denote differences between days within treatments. N = 12.

Cu conc (mg l^{-1})	CO_2 assimilation (umol $m^{-2}s^{-1}$)	Internal CO_2 (ul l^{-1})
0.06 (control)	2.32 a	351.37 a
1.0	1.51 b	351.77 a
2.0	1.12 c	350.92 a
2.5	1.10 c	350.92 a
3.0	1.01 c	350.06 a

control (Table 3). However, treatments receiving 2.0, 2.5, and 3.0 mg l^{-1} did not differ. Furthermore, there was a 25.8, 27.1, and 33.1% increase in CO_2 assimilation in plants grown at 1.0 mg l^{-1} Cu when compared to that obtained in plants receiving higher Cu concentrations. Internal CO_2 concentrations were not different among plants grown in all treatments (Table 3).

After seven days of growth, Cu accumulation was significantly higher in plants receiving 1.0 to 3.0 mg l^{-1} Cu in comparison to the control (Table 4). Furthermore, Cu accumulation in *Salvinia* grown at 1.0 mg l^{-1} was significantly lower than those plants receiving higher concentrations. At the end of 14 days, *Salvinia* still showed an increase of Cu uptake correlated with increasing Cu concentrations in the growth media. However, examining the individual treatments showed that at 1.0 mg l^{-1} Cu, *Salvinia* accumulation was similar to that of the 0.06 and 2.0 mg l^{-1} concentration. Copper uptake of the media containing 3.0 mg l^{-1} was the highest in comparison to the other treatments with a 43.9% increase in comparison to the nearest treatment, 2.5 mg l^{-1}. Whereas, Cu accumulation in *Salvinia* was similar in those plants grown at 2.0 and 2.5 mg l^{-1}. With the exception of those plants grown at 0.06 and 3.0 mg l^{-1} Cu, *Salvinia* uptake of Cu was significantly higher during the first seven days in comparison to 14 days of the experiment (Table 4). These results coincide with the BCF calculation of each treatment after 14 days of Cu exposure that was nearly 20-fold higher in plants at 3.0 mg l^{-1} Cu in comparison to the control and twice as high in plants grown in 2.5 mg l^{-1} Cu (Table 4).

DISCUSSION

The association between reduced growth and increased Cu concentration (Table 1) has also been observed in several other aquatic plants such as *Lemna minor* L. (Teisseire *et al.*, 1998), *Potamogeton pectinatus* L., *Vallisneria spiralis* L., *Hydrilla verticillata* (L.f.) Royle (Guilizzoni 1991), and *Elodea nuttallii* (Planch.) St. John (Van der Werff and Pruyt, 1982). Sarkar and Jana (1986) reported that *Azolla pinnata* R. Br. growth was significantly reduced following exposure to 2.0 mg l^{-1} Cu, whereas plants at 1.0 mg l^{-1} exhibited growth similar

TABLE 4. Cu uptake (μg Cu g^{-1} d. wt) in *Salvinia* after seven and 14 days of exposure to varying concentrations. LSD (P = 0.05) = Least Significant Difference value for difference between means within a column. Same letter denotes no statistical difference. Lower case letters denote differences between treatments within a day and upper case letters denote differences between days within treatments. N = 12. Bioconcenration factor (BCF) was determined as the ratio of Cu concentration in the plant tissue relative to the concentration in the external media after 14 days of exposure. N = 6.

	Length of exposure (days)		
Cu (mg l^{-1})	7	14	BCF
0.06 (control)	1.50.35 aA	167.33 aA	308.91 a
1.0	1833.54 bA	735.62 abA	1390.08 ab
2.0	2934.90 cA	1545.49 bcB	2132.87 bc
2.5	3111.67 cA	2413.75 cB	3111.63 c
3.0	3519.20 cA	4304.44 dB	4304.44 d

to the control after 28 days of incubation. A possible explanation of decreasing *Salvinia* growth with increasing Cu concentrations might be attributed to Cu-induced ethylene production. Matoo *et al.*, (1986) reported that intercellular membrane and organelle disintegration in giant duckweed (*Spirodela oligorrhiza* (Kurz) Hegelm) resulted from induced ethylene production by copper. In our study, reduction in *Salvinia* growth was directly related to a decline in CO_2 assimilation as a function of Cu increase (Table 3). The negative impact of increasing Cu concentration on CO_2 assimilation was reported to be due to the inhibitory effect on the electron transport of photosystem II (PS II) (Sarkar and Jana, 1986; Renganathan and Bose, 1989). The decline in PS II was attributed to degradation and leakage of the chloroplast membrane induced by Cu (Ouzounidou *et al.*, 1993). Furthermore, the decline in CO_2 assimilation might be influenced by the reduction in photosynthetic pigments, which was associated with increasing Cu concentrations (Table 2). A reduction in photosynthetic pigment concentration in *Salvinia* has been associated with an increase in metal contamination (Gardner and Al-Hamdani, 1997; Nichols *et al.*, 2000). Sarkar and Jana (1986) attributed the reduction in chlorophyll concentration to the influence of Cu on declining chlorophyllase activity. Furthermore, the decline in CO_2 assimilation and photosynthetic pigment might be related to membrane destruction by lipid peroxidation, which was found to be associated with an increase in Cu accumulation (Halliwell and Gutteridge 1984). Mattoo *et al.* (1986) reported that free radical formation was induced by an increase in Cu concentration that later reformed into H_2O_2. As a defense mechanism against increasing free radicals, plants usually respond by increasing catalase activity (Foyer *et al.*, 1994). Catalase activity was found to decline gradually in duckweed as Cu concentrations increased above 0.2 mg l^{-1} in the nutrient media (Teisseire *et al.*, 1998).

Copper uptake by *Salvinia* was significantly higher with an increase in Cu concentration in the growth media (Table 4). With the exception of those plants grown in 3.0 mg l^{-1} Cu, the concentration of Cu in *Salvinia* was significantly higher at the end of seven days of plant growth in comparison to that at day 14.

In calculating Cu uptake as μg g^{-1} plant dry weight, growth should be considered a factor in interpreting the values at each individual treatment. This conclusion equally reflects Cu uptake in plants grown in 3.0 mg l^{-1} Cu, where growth was severely affected at the end of seven days with a chlorotic and necrotic appearance that advanced with time. However, the BCF was highest for the plants grown in 3.0 mg l^{-1} Cu followed by a decreasing order as the Cu concentration decreased in the media. In comparison with other species, *Salvinia* uptake of Cu was comparable to that obtained with monkey flower (*Mimulus guttatus* DC.; Tilstone and MacNair, 1997) and iceplant (*Mesembryanthemum crystallinum* L.; Thomas *et al.*, 1998) using equivalent Cu concentrations and incubation periods. In addition, *Salvinia* has the ability to survive and grow under highly eutrophic environments unsuitable for other species found in similar environments such as *Azolla* and duckweed (Reddy and DeBusk, 1985). This can be used as an additional indication that *Salvinia* can be considered an essential agent in phytoremediation.

In conclusion, this study demonstrated that increases in Cu concentration from 1.0 to 3.0 mg l^{-1} negatively impacted plant growth, photosynthetic pigments, and CO_2 assimilation. However, the reduction in plant growth was not severe enough to totally inhibit plant growth even at Cu concentrations of 3.0 mg l^{1}. *Salvinia* demonstrated the ability to accumulate significant concentrations of Cu in its tissues. This, in addition to its high growth rate and ease in harvesting, make it a possible candidate for phytoremediation. However, further research should be implemented to investigate the performance of *Salvinia* under field conditions.

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LITERATURE CITED

BERVOETS, L., L. PANIS and R. VERHEYEN. 1994. Trace metal levels in water, sediments, and *Chironomus* GR. Thurni from different water courses on flanders (Belgium). Chemosphere 29:1591–1601.

BOWYER, J. R. and R. C. LEEGOOD. 1997. In, P. M. Dey and J. B. Harborne (eds.). *Plant Biochemistry*. Academic Press, Inc., San Diego, CA.

BOYD, C. E. 1990. Water quality in ponds for aquaculture. Birmingham Publishing Company, Birmingham, AL.

BUCK SCIENTIFIC. 1996. *Buck model 210 vgp atomic absorption spectrophotometer operating manual*. Buck Scientific, Inc. East Norwalk, CT.

CHANEY, R. L. 1970. Effect of nickel on iron metabolism by soybean. Ph. D. Dissertation, Purdue University, Lafayette, Ind. Diss. Abstr. Int. 31:1692–93.

DEBUSK, W. F. and K. R. REDDY. 1987. Growth and nutrient uptake potential of *Azolla caroliniana* Willd. and *Salvinia rotundifolia* Willd. as a function of temperature. Environ. Exp. Bot. 27:215–221.

DOONG, R. L., G. E. MACDONALD and D. G. SHILLING. 1993. Effect of fluoridone on chlorophyll, carotenoid, and anthocyanin content of *Hydrilla*. J. Aquat. Plant Manag. 31:55–59.

FOYER, C. H., M. LELANDAIS and K. KUNERT. 1994. Photooxidative stress in plants. Physiol. Plant. 92:696–717.

GARDNER, J. L. and S. H. AL-HAMDANI. 1997. Interactive effects of aluminum and humic substances on *Salvinia*. J. Aquat. Plant Manag. 35:30–34.

GUILIZZONI, P. 1991. The role of heavy metals and toxic materials in the physiological ecology of submerged macrophytes. Aquat. Bot. 41:87–109.

HALLIWELL, B. and J. M. C. GUTTERIDGE. 1984. Oxygen toxicity, oxygen radicals, transition metals, and diseases. Biochem. J. 219:1–4.

HAWF, L. R. and W. E. SCHMID. 1967. Uptake and translocation of zinc by intact plants. Plant Soil 27:249–260.

HOAGLAND, D. R. and D. I. ARNON. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agri. Exp. Stn. Cir. No. 347:1–32.

INSKEEP, W. P. and P. R. BLOOM. 1985. Extinction coefficients of chlorophyll *a* and *b* in N, N-dimethylformamide and 80 % acetone. Plant Physiol. 77:483–485.

LEE, C. L., T. C. WANG, C. H. HSU and A. A. CHIOU. 1998. Heavy metal sorption by aquatic plants in Taiwan. Bull. Environ. Contam. Toxicol. 61:497–504.

LEWIS, M. A. 1993. Freshwater primary producers. Pp. 28–50 in, P. Calow (ed.). *Handbook of ecotoxicology vol. 1.* Blackwell Scientific, Oxford, England.

LINDER, M. C. 1991. *Biochemistry of copper.* Plenum Press, New York, N.Y.

LINGLE, J. C., L. O. TIFFIN and J. C. BROWN. 1963. Iron uptake-translocation of soybean as influenced by other cations. Plant Physiol. 38:71–76.

MARSCHNER, H. 1995. *Mineral Nutrition of Higher Plants. 2nd ed.* Academic Press, New York, NY.

MATTOO, A. K., J. E. BAKER and H. E. MOLINE. 1986. Induction by copper ions of ethylene production in *Spirodela oligorhiza*: Evidence for a pathway independent of 1-aminocyclopropane-1-carboxylic acid. J. Plant Physiol. 123:193–202.

MCDERMITT, D. K., J. M. NORMAN, J. T. DAVIS, J. BALL, T. J. ARKEBAUER, J. M. WELLES and S. R. ROEMER. 1989. CO_2 response curves can be measured with a field-portable closed-loop photosynthesis system. Ann. Sci. For. 46:416–420.

MOORE, J. W. 1991. *Inorganic contaminants in surface water.* Springer-Verlag, New York, NY.

MORETTI, A. and G. S. GIGLIANO. 1988. Influence of light and pH on growth and nitrogenase activity on temperate-grown azolla. Biol. Fertil. Soils 6:131–136.

NAUMAN, C. E. 1993. Salviniaceae. Pp. 336–337, in Flora North America Editorial Committee. *Flora of North America vol. 2. Pteridophytes and Gymnosperms.* Oxford University Press, Oxford.

NICHOLS, P. B., J. D. COUCH and S. H. AL-HAMDANI. 2000. Selected physiological responses of *Salvinia minima* to different chromium concentration. Aquat. Bot. 1439:18.

OLGUIN, E. J., E. HERNANDEZ and I. RAMOS. 2002. The effect of both different light condition and the pH value on the capacity of *Salvinia minima* Baker for removing cadmium, lead and chromium. Acta Biotechnol. 22:121–131.

OUZOUNIDOU, G., R. LANNOYE and S. KARATAGLIS. 1993. Photoacoustic measurements of photosynthetic activities in intact leaves under copper stress. Plant Sci. 89:221–226.

REDDY, K. R., and W. R. DEBUSK. 1985. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: *Azolla*, duckweed, and *Salvinia*. Econ. Bot. 39:200–208.

RENGANATHAN, M. and S. BOSE. 1989. Inhibition of primary photochemistry of photosystem II by COPPER IN ISOLATED PEA CHLOROPLASTS. BIOCHIMICA ET BIOPHYSICA ACTA. 974:247–253.

SALOMONS, W., U. FORSTNER and P. MADER. 1995. *Heavy metals: Problems and Solutions.* Spring Publishing, Berlin.

SALT, D. E., R. D. SMITH and I. RASKIN. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:643–68.

SARKAR, A. and S. JANA. 1986. Heavy metal pollutant tolerance of *Azolla pinnata.* Water, Air, and Soil Pollut. 27:15–18.

SPACIE, A., L. S. MCCARTY and G. M. RAND. 1995. Bioaccumulation and bioavailability in multiphase systems. P. 493 in *Fundamentals of Aquatic Toxicology*. G. M. Rand (ed.). Taylor and Francis, Washington.

STEEL, R. G. D. and J. D. TORRIE. 1980. *Principles and procedures of statistics: a biometrical approach.* McGraw-Hill, New York, NY.

TEISSEIRE, H., M. COUDERCHET and G. VERNET. 1998. Toxic responses and catalase activity of *Lemna minor* L. exposed to folpet, copper, and their combination. Ecotox. Env. Saf. 40:194–200.

THOMAS, J. C., F. K. MALICK, C. ENDRESZL, E. C. DAVIES and K. S. MURRAY. 1998. Distinct responses to copper stress in the halophyte *Mesembryanthemum crystallinum*. Physiol. Plant. 102:360–368.

TILSTONE, G. H. and M. R. MACNAIR. 1997. The consequence of selection for copper tolerance on the uptake and accumulation of copper in *Mimulus guttatus*. Ann. Bot. 80:747–751.

VAN DER WERFF, M. and M. J. PRUYT. 1982. Long-term effects of heavy metals on aquatic plants. Chemosphere 11:727–739.

VAVILIN, D. V., V. A. POLYNOV, D. N. MATORIN and P. S. VENEDIKTOV. 1995. Sublethal concentrations of copper stimulate photosystem II photoinhibition in *Chlorella pyrenoidosa*. J. Plant Physiol. 146:609–614.

WALLACE, A., and P. C. DEKOCK. 1966. Translocation of iron in tobacco, sunflower, and bush bean plants. Pp. 3–9, in A. Wallace (ed.). *Current Topics in Plant Nutrition*. Edward Bros., Ann Arbor.